Innate Immunity in the Lungs

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Innate immunity is a primordial system that has a primary role in lung antimicrobial defenses. Recent advances in understanding the recognition systems by which cells of the innate immune system recognize and respond to microbial products have revolutionized the understanding of host defenses in the lungs and other tissues. The innate immune system includes lung leukocytes and also epithelial cells lining the alveolar surface and the conducting airways. The innate immune system drives adaptive immunity in the lungs and has important interactions with other systems, including apoptosis pathways and signaling pathways induced by mechanical stretch. Human diversity in innate immune responses could explain some of the variability seen in the responses of patients to bacterial, fungal, and viral infections in the lungs. New strategies to modify innate immune responses could be useful in limiting the adverse consequences of some inflammatory reactions in the lungs.

Keywords: endotoxin; innate immunity; lung inflammation; Toll-like receptors

The alveolar membrane is the largest surface of the body in contact with the outside environment. Like the skin and the gastrointestinal mucosa, the lungs are continuously exposed to a diverse array of microbes and organic and inorganic particulate materials. As life evolved, strategies were needed to recognize material from the outside environment, and to distinguish potentially harmful agents from most innocuous foreign material. Higher vertebrates have developed two interactive protective systems: the innate and adaptive immune systems. The innate immune system is older and consists of soluble proteins, which bind microbial products, and phagocytic leukocytes resembling primitive amebae, which float through the bloodstream and migrate into tissues at sites of inflammation, or reside in tissue waiting for foreign material. The innate immune system is always active and is immediately responsive, ready to recognize and inactivate microbial products entering the lungs and other tissues. Its specificity is relatively broad, and based on the recognition of common microbial motifs. Higher animals have evolved an adaptive immune system of lymphocytes that respond specifically to signals from the innate immune system by producing high-affinity antibodies to very specific peptide sequences presented on specialized antigen-presenting cells. These antibodies opsonize microbes and viruses and facilitate their destruction by leukocytes in tissue and lymph nodes. Cytokines and growth factors produced by macrophages and dendritic cells of the innate immune system drive the specialized antibody responses

of the adaptive immune system. The adaptive immune system has a memory component lacking in the innate immune system. Together, the innate and the adaptive immune systems enable the host to react to the array of microbial and other products encountered in everyday life.

This article provides an overview of new developments in innate immunity and shows their relevance for lung antimicrobial defenses.

INNATE IMMUNITY

The innate immune system includes soluble proteins that bind to microbial products and leukocytes that ingest particulates and kill microorganisms (Table 1) (1). The understanding of innate immunity began in 1774, when leukocytes were first identified at sites of inflammation (reviewed in Reference 2). Over 100 yr later, in 1882, Metchnikov identified mobile ameboid cells in sea anemones that could ingest particulate dyes, and similar cells in water fleas that could engulf fungal spores. Suspecting that these cells might have a defensive function, he inserted a rose thorn into a starfish larva to show that the mobile cells accumulated around the foreign body. He named these cells phagocytes, meaning “devouring cells” in Greek. In addition, a series of host proteins have evolved that bind bacteria and their products, and facilitate recognition by receptor complexes on the surface of leukocytes and other cells. The modern understanding of innate immunity accelerated dramatically with recent discoveries about proteins that bind bacterial products, and the receptor systems used by leukocytes to recognize bacterial, fungal, and viral products.

It has been known for many years that circulating proteins are important in the recognition of bacterial products by leukocytes. For example, complement components bind to bacterial cell walls and facilitate bacterial uptake by leukocytes by the C3b receptor. Serum is an excellent opsonin that promotes phagocytosis of bacteria by neutrophils, and heat inactivation, which destroys complement, eliminates this opsonic effect. The complement component C5a is a potent chemotactic factor that interacts with a specific receptor on neutrophils to facilitate directed migration of neutrophils toward sites of inflammation (3). The collectins are a group of related proteins that serve as opsonins for bacterial products in plasma and tissues. The collectins have a common structure, including a globular-like domain and a collagen-like tail, and include mannose-binding protein in serum, conglutinin, and the lung surfactant proteins SP-A and SP-D. Binding of collectins to the bacterial surface promotes phagocytosis by macrophages and neutrophils. Mannose-binding protein recognizes mannose on the bacterial surface, and activates the alternate complement pathway, leading to the accumulation of C3b on the microbial surface. Children with mannose-binding protein deficiency have defective opsonic function in serum despite normal levels of immunoglobulins and complement (4).

A great deal has been learned from the study of LPS binding protein (LBP), a prototypical protein that prepares LPS shed from the outer membrane of gram-negative bacteria for recognition by specific protein receptor complexes on leukocytes. LBP

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TABLE 1. COMPONENTS OF THE INNATE IMMUNE SYSTEM

	Afferent (Recognition)	Efferent (Effector)
Humoral (soluble factors)	LBP, CD14, collectins, surfactant proteins, properdin, C3b, pentraxins	Cytokines, antimicrobial peptides, lysozyme, BPI, complement, lactoferrin, acute-phase reactants
Cellular (macrophages, PMNs)	TLRs, CD14, FMLP receptor, NOD1, NOD2, dectin-1	Antimicrobial peptides, proteases, lipases, glycosidases, H ₂ O ₂ , myeloperoxidase and toxic oxygen species, nitric oxide, peroxynitrite

Definition of abbreviations: BPI = bactericidal permeability enhancing factor; FMLP = formylmethionylleucylphenylalanine; LBP = LPS binding protein; NOD = nucleotide binding, oligomerization domains; PMN = neutrophil; TLR = Toll-like receptor. Modified from Reference 1.

is produced in the liver as an acute-phase reactant, circulates in plasma in relatively high concentrations ($\mu\text{g/ml}$), and is widely distributed in tissue fluids. LBP binds the lipid A portion of LPS with high affinity (K_d in the nanomolar range) and 1:1 stoichiometry, and solubilizes LPS aggregates to form stable LBP:LPS complexes that are extremely potent in cellular activation (5). LPS also binds to high-density lipoproteins in plasma with relatively high affinity, and in this form LPS is biologically inactive (6). LPS also binds nonspecifically to other constituents of plasma, including albumin, immunoglobulins, and other proteins, but there is no evidence that this affects the biologic activity of LPS. Although LBP is derived primarily from the liver, extrahepatic production of LBP has been reported, including production by pulmonary artery smooth muscle cells and type II pneumocytes in alveolar walls (7, 8).

Following the description of LBP, a search began to determine whether LBP:LPS complexes interacted with a specific receptor on leukocytes. Studies using LBP-coated erythrocytes and a panel of blocking monoclonal antibodies established that leukocytes recognize LBP:LPS complexes by the CD14 receptor on the cell surface (9), which was originally described as a monocyte differentiation antigen. As mononuclear cells mature, they express increasing amounts of CD14, and at the same time, they become steadily more responsive to LBP:LPS complexes (10). CD14 is anchored in the cell membrane by a glycosylphosphatidylinositol group and is shed from the cell membrane where it accumulates in tissue fluids and plasma. LBP transfers LPS to soluble CD14 and soluble LPS:CD14 complexes mediate LPS responsiveness by endothelial cells and other cells that do not bear much membrane CD14 (11). Soluble CD14 also enhances the binding of LPS to high-density lipoproteins, so plasma proteins can both enhance and reduce the bioactivity of LPS in plasma (12). Soon after the discovery of the role of CD14 in recognizing bacterial LPS it was recognized that CD14 was involved in the recognition of other bacterial products, and CD14 was designated as a pattern-recognition receptor (i.e., a receptor that recognizes common bacterial pattern motifs) (13). The observation that the membrane anchor portion of CD14 was not required for LPS-dependent cellular activation led to a search for one or more additional proteins that interact with CD14 to transduce signals into the cell.

The discovery of LBP in human plasma and the recognition of the critical role of the CD14 receptor for cellular activation by LPS were important clues to understanding microbial recognition mechanisms by the innate immune system. The next critical discovery came from the study of immunity in fruit flies. A family of proteins recognized to be important in embryonic dorsal ventral development in *Drosophila* were found to have a role in

antifungal defenses (14). Medzhitov and colleagues found that one of these proteins, *Drosophila* Toll, had a human homolog (human Toll) that was a type 1 transmembrane protein whose intracellular portion was highly homologous with the interleukin (IL)-1 receptor (15). Activation of the human Toll molecule led to activation of nuclear factor (NF)- κ B and the production of a variety of proinflammatory cytokines. At almost the same time, Poltorak and colleagues, using a genetic approach, discovered that mice naturally resistant to LPS had a mutation in the intracellular portion of a protein that was identical to murine Toll-4, and this established that Toll-4 was the signaling portion of the LPS receptor complex (16).

Subsequent studies showed that an additional protein, designated MD-2, was needed for full activation of Toll-4 by LBP:LPS complexes and membrane CD14 (17). The intracellular adaptor protein, MyD88, which was known to be important in intracellular signaling by the IL-1 receptor, was shown to be critical for TLR4-dependent signaling in response to LPS (18). Interestingly, the Toll receptors do not promote phagocytosis, but function in the membrane of the developing phagosome as receptors that sense what is being ingested, and initiate intracellular signaling (19). The importance of Toll-like receptors (TLRs) for innate immunity in human lungs was established when Arbour and colleagues found that humans with mutations in the extracellular portion of TLR4 were hyporesponsive to inhaled LPS (20). Subsequently, humans with TLR4 mutations were shown to be relatively protected against progression of atherosclerosis, providing a role for innate immunity in vascular disease (21).

Ten human TLRs have been identified in humans and a number of important principles have emerged. The first is that the TLRs recognize an array of bacterial, fungal, and viral products, including structural molecules in the microbial cell wall like LPS and lipoteichoic acid, secreted proteins like lipoproteins, unmethylated bacterial DNA, and double-stranded viral RNA (Table 2). For example, TLR2 recognizes gram-positive lipoteichoic acids, TLR4 recognizes gram-negative LPS, TLR5 recognizes flagellin, and TLR9 recognizes unmethylated bacterial DNA. A second principle is that cooperativity among TLRs provides a combinatorial mechanism to cope with the array of microbial products in nature. Although single TLRs recognize some bacterial products, TLRs combine together to increase the diversity of bacterial ligands that can be sensed by host cells.

A third principle is that all of the known TLRs signal via their intracellular tails by activating a cascade of intracellular kinases, leading to diverse gene expression. The intracellular portion of each known TLR contains a TIR domain (Toll-IL-1 receptor motif). Five different cytoplasmic adaptor proteins bind

TABLE 2. HUMAN TOLL-LIKE RECEPTORS AND THEIR LIGANDS

Receptor	Ligands	Animal Species
TLR-1	Bacterial lipopeptides	Human, mouse
TLR-2*	Lipopeptides, lipoteichoic acid, peptidoglycan	Human, mouse
TLR-3	Double-stranded RNA, polyinosine:cytosine	Human, mouse
TLR-4	LPS	Human, mouse
TLR-5†	Flagellin	Human, mouse
TLR-6	Zymosan, lipopeptides	Human, mouse
TLR-7†	Single-stranded viral RNA, imiquimod	Human, mouse
TLR-8†	Single-stranded viral RNA, imiquimod	Human, mouse
TLR-9†	Unmethylated CpG DNA	Human, mouse
TLR-10	Uncertain	Human
TLR-11, TLR-12, TLR-13	Uncertain	Mouse

Definition of abbreviation: TLR = Toll-like receptor.

* TLR-2 cooperates with TLR-1 and TLR-6 to recognize lipoteichoic acid, zymosan, and other ligands.

† TLR-5, TLR-7, TLR-8, and TLR-9 cooperate to recognize CpG DNA, ssRNA, and flagellin.

Modified from References 1 and 70.

to the different TLRs and initiate intracellular signaling. These include myeloid differentiation factor 88 (MyD88), MyD88 adaptor-like protein (MAL) (Tirap), Toll receptor-associated activator of interferon (TRIF) (Ticam-1), MyD88-4 (Toll receptor-associated molecule [TRAM]), and MyD88-5 (1). The signaling pathway involves recruitment and activation of IL-1 receptor-associated kinase (IRAK)-4, which phosphorylates IRAK-1 and IRAK-2. Activation of TNF receptor-associated factor (TRAF)-6 and TGF- β activated kinase (TAK)-1 leads to phosphorylation of I-kappa kinase (IKK)- γ and the phosphorylation and degradation of I κ B, resulting in translocation of NF- κ B to the nucleus and the transcription of a large number of proinflammatory and antiinflammatory gene products. IRAK-4 and TAK-1 also activate p38 mitogen-activated protein kinase and c-Jun N-terminal kinase (JNK), leading to broad intracellular kinase activation.

Signaling through some TLRs, like TLR4, is enhanced by additional membrane proteins like CD14 and MD-2. The MD-2 protein is an essential component of the CD14-TLR4 signaling complex that is expressed in many different tissues, including the lungs (22). Like CD14, MD-2 is shed into plasma and circulating MD-2 is increased in patients with sepsis (23). The MyD88 intracellular adaptor mediates signaling through all of the known TLR receptors except TLR3, although its importance for individual TLRs varies. Mice deficient in MyD88 are highly susceptible to bacterial infections, particularly gram-negative infections (24). A fourth important principle is that membrane TLRs are not limited to leukocytes, but are found on the surface of somatic cells, such as airway and alveolar epithelial cells. The presence of TLR receptors on nonmyeloid cells broadens the cellular diversity of the innate immune system.

Beutler and coworkers have used "forward genetics" in mice to identify key components of the signaling pathways for the known murine TLRs (25). Germline mutations have been induced in mice by exposure to the mutagen *N*-ethyl-*N*-nitrosourea. By inbreeding progeny, rare nonlethal mutations in innate immunity pathways have been identified by exposing peritoneal macrophages to known TLR agonists and measuring tumor necrosis factor α (TNF- α) in a high throughput assay. This approach has led to the discovery of a number of key intermediates in TLR signaling, including two different pathways that diverge from TLR-4, designated LPS-1 and LPS-2. These two pathways are believed to account for all cellular activation by LPS (Figure 1) (26). The LPS-1 pathway is the classical LPS signaling pathway in leukocytes and is mediated by the MyD88 intracellular adaptor molecule. The LPS-1 pathway activates the enzyme intermediates of the IL-1 receptor pathway, including

IRAK4 and IRAK1, and leads to rapid NF- κ B activation with the production of an array of proinflammatory cytokines, including IL-1, IL-6, IL-8, and the counterregulatory cytokine IL-10. By contrast, the LPS-2 pathway is mediated by different intracellular adapter proteins named TRAM and TRIF and produces slower and sustained activation of interferon (IFN) response elements with the production of Type I IFNs and the induction of inducible nitric oxide synthase. Although many of the intracellular signaling events are common among the various TLRs, increasing evidence suggests that important differences in intracellular signaling pathways exist. An important question is whether relatively specific inhibitors can be developed that block or dampen signaling through some TLR pathways without affecting others.

The innate immune system has a critical role in activating and coordinating the adaptive immune system (reviewed in Reference 27). Macrophages and dendritic cells in tissue process microbial antigens and present them in association with class I and class II molecules to responding T lymphocytes. Macrophage-derived IL-1 promotes lymphocyte proliferation, and IL-6 promotes B-cell growth and antibody production. The complement cascade and natural killer cells provide non-TLR-dependent mechanisms to activate adaptive immunity. In addition, the NOD proteins (nucleotide binding, oligomerization domains) serve as intracellular sensors of gram-positive peptidoglycans and directly activate NF- κ B and the production of Type I IFNs (IFN- α and IFN- β) (28). LPS and other microbial products have well-known adjuvant effects for adaptive immunity, enhancing antibody production in response to microbial antigens. It seems that the LPS-2 pathway mediates the adjuvant effect of LPS, suggesting that the adjuvant effect of LPS is not completely dependent on the cytokine responses induced by LPS (29).

INNATE IMMUNITY IN THE LUNGS

Innate immune mechanisms defend the air spaces from the array of microbial products that enter the lungs on a daily basis and are evident from the nasopharynx to the alveolar membrane. Large particles deposit in the nasopharynx and tonsillar regions when inertial forces carry them out of the bending airstream and against the posterior pharyngeal wall. Particles that are carried into the conducting airways sediment onto the mucociliary surface of the airways under the influence of gravity, where they encounter soluble constituents in airway fluids and the upward propulsive force of the mucociliary system. Particles 1 μ m in size and smaller, the size of bacteria and viral particles, are carried to the alveolar surface where they interact with soluble

Figure 1. Simplified diagram of some Toll-like receptor (TLR) signaling pathways. Flagellin binds to TLR5; lipoproteins bind to cooperative clusters of TLR2, -1, and -6; LPS binds to the complex formed by CD14, MD-2, and TLR4; and bacterial DNA binds to TLR9, which is located in the membrane of phagolysosomes. MyD88 has a key role in the proximal signaling by these TLRs. Two LPS signaling pathways diverge from TLR4, LPS-1 and LPS-2. The LPS-1 pathway is mediated by MyD88 and through IRAK-4 and IRAK-1 leads to nuclear factor (NF)-κB activation and cytokine production. The LPS-2 pathway is mediated by TRAM and TRIF and leads to the production of Type I interferons and nitric oxide, and has a role in the adjuvant effect of LPS in adaptive immune responses. A more complete signaling map can be found in Reference 1.

components in alveolar fluids (e.g., IgG, complement, surfactant, and surfactant-associated proteins) and alveolar macrophages (Figure 2). Normally, alveolar macrophages account for approximately 95% of airspace leukocytes, with 1 to 4% lymphocytes and only about 1% neutrophils, so that the alveolar macrophage is the sentinel phagocytic cell of the innate immune system in the lungs. Other cells in the airways and alveolar environment can sense microbial products, because pattern recognition recep-

tors in the TLR family are found on alveolar walls and the ciliated epithelium of the conducting airways (Figure 3).

The soluble constituents of airway and alveolar fluids have an important role in innate immunity in the lungs. In the conducting airways, constituents of airway aqueous fluids include lysozyme, which is lytic to many bacterial membranes; lactoferrin, which excludes iron from bacterial metabolism; IgA and IgG; and defensins, which are antimicrobial peptides released from leukocytes and respiratory epithelial cells (30, 31). IgG is the most abundant immunoglobulin in alveolar fluids, and complement proteins and surfactant-associated proteins serve as additional microbial opsonins. In particular, SP-A and SP-D are members of the collectin family and promote phagocytosis of particulates by alveolar macrophages. Alveolar surfactant lipids and SP-A and SP-D bind LPS and prevent its interaction with LBP in alveolar fluids and the CD14:TLR4 complex on alveolar macrophages (32, 33). Alveolar fluids contain high concentrations of LBP and soluble CD14 (sCD14), which are key molecules in the recognition of LPS by alveolar macrophages and other cells in the alveolar environment (34, 35). LBP and sCD14 have molecular weights of approximately 60 and 50 kD, respectively, and probably diffuse from the plasma compartment into alveolar fluids much like albumin (molecular weight, 67 kD). LBP can be produced locally by type II pneumocytes, however, and LBP production has been reported in pulmonary artery smooth muscle cells *in vitro* (7, 8, 36). Soluble CD14 is released from the surface of alveolar macrophages by proteases, and this is enhanced by IL-6, which is abundant in the bronchoalveolar lavage (BAL) fluids of patients with lung injury (37–39). Blood monocytes and newly recruited monocyte-macrophages express considerably more membrane CD14 than mature alveolar macrophages, so newly recruited cells are likely to be an additional source of soluble CD14 in alveolar fluids (40).

Alveolar macrophages are avidly phagocytic and ingest all types of inhaled particulates that reach the alveolar spaces.

Figure 2. The alveolar environment in the lungs. Scanning electron micrograph of a rat lung showing the images of erythrocytes in the alveolar wall capillaries and two ruffled alveolar macrophages (MP) in the alveolar space (ALV). Alveolar macrophages make up approximately 95% of the leukocytes in the airspaces of human lungs, and 100% of the leukocytes in the lungs of pathogen-free mice.

Figure 3. TLR2 and CD14 in the lungs of a rabbit. TLR2 is labeled *red* and CD14 is labeled *green*. Colocalization of TLR2 and CD14 is shown in *yellow*. TLR2 is visible on the alveolar epithelium and on alveolar macrophages in the airspace. CD14 is visible on alveolar macrophages, and neutrophils in the intravascular and alveolar space. The *bright yellow* alveolar macrophage shows high levels of expression of both TLR2 and CD14. Similar results are found when the sections are labeled for TLR4 and CD14.

Remarkably, one of the primary roles of the alveolar macrophage is to keep the airspaces quiet, and they ingest large numbers of inert particulates like amorphous silicates and carbon-graphite particles without triggering inflammatory responses. When bacteria are opsonized by IgG, complement, or SP-A and SP-D in the airspaces, they are ingested by alveolar macrophages and the TLRs in the phagosomal membrane provide discrimination among the various microbial products entering the cell (19). The proinflammatory cytokines produced by macrophages, notably IL-8 and related CXC chemokines, initiate a localized inflammatory response by recruiting neutrophils from the lung capillary networks into the alveolar space. Alveolar macrophages are poor antigen-presenting cells, but carry microbial antigens into the interstitium and to regional lymph nodes where they are taken up by specialized dendritic cells and presented to responding lymphocytes to initiate adaptive immune responses. Alveolar macrophages also have an important role in producing CC chemokines, such as MCP-1 and RANTES (regulated on activation, normal T-cell expressed and secreted), which recruit activated monocytes and lymphocytes into sites of inflammation in the lungs.

Another important principle is that acute inflammation alters the set point for the induction of innate immune reactions in the lungs. Normally, the airspace environment is a relatively quiet place despite the array of microbial and other products that enter the airspaces by inhalation or subclinical oropharyngeal aspiration. Surfactant lipids and proteins are present in very high concentrations as compared with LBP and sCD14. Surfactant lipids, and SP-A and SP-D, bind LPS and reduce its biological effects (41). When inflammation occurs, the concentrations of

SP-A and SP-D fall, whereas the concentrations of LBP and sCD14 rise markedly, enhancing the effects of LPS in lung fluids (42, 43). TLRs are expressed on alveolar and airway epithelial cells, but the responsiveness of these cells to LPS is limited because of low expression of membrane MD-2 (22, 44). CD14 is shed from macrophage membranes at sites of inflammation, in part by the action of proteases like matrix metalloproteinase (MMP)-9 and MMP-12 (37, 45). Innate immune responses in the airspaces initiate local inflammatory responses, and these inflammatory responses change the threshold for subsequent inflammatory responses to bacterial products entering the lungs.

Recent studies have defined an important role for airway epithelial cells in innate immune responses in the lungs (46). The airway epithelium restrains the growth of microbes in the conducting airways by several different mechanisms. The ciliated epithelial cells move fluid, mucus, and trapped particulates upward and out of the lungs. Airway fluids contain soluble proteins that contain bacterial growth, including lysozyme, lactoferrin, and the antimicrobial defensins. Airway epithelial cells express low levels of CD14 and TLR1-6 and -9, and sense bacteria in the mucociliary fluid by the same TLR-dependent mechanisms used by leukocytes. Engagement of pattern recognition receptors enhances the production of antimicrobial defensins by airway epithelial cells, and stimulates epithelial cells to produce CXC and CC chemokines, which recruit neutrophils into the airway lumen (31). Airway epithelial cells produce IL-1 β , IL-6, IL-8, RANTES, granulocyte-macrophage colony-stimulating factor, and transforming growth factor β , in addition to other proinflammatory cytokines (46). Airway epithelial cells also recognize unmethylated bacterial DNA by membrane TLR-9, leading to NF- κ B activation and production of IL-6, IL-8, and β 2-defensin in the airways (47). Skerrett and coworkers have shown that mice expressing a dominant negative I κ B construct in distal airway epithelial cells, which prevents NF- κ B activation, do not recruit PMNs normally into the airways in response to inhaled LPS (48). This experiment verifies the importance of LPS recognition by distal airway epithelial cells *in vivo*, and shows that epithelium-derived cytokines are probably just as important as macrophage-derived cytokines in driving innate inflammatory responses in the airspaces. Alveolar macrophages probably have a considerable amount of help in initiating innate immune responses in the airspaces.

Despite the critical role of TLR family members in recognition of bacterial products *in vitro*, lung innate immune responses *in vivo* are very complex and more progress is needed in translating discoveries about innate immunity in simplified laboratory systems to lung antimicrobial defenses *in vivo*. Lung inflammatory gene expression in response to LPS is regulated by TLR4, and TLR4 mediates virtually all of the observed gene expression in response to inhaled gram-negative bacteria in mice (49). Although TLR4-deficient mice have blunted inflammatory responses to inhaled LPS, the clearance of live *Escherichia coli* from the lungs over 6 h is normal, supporting the redundancy of antibacterial defense mechanisms in the lungs (50). Furthermore, although the MyD88 adaptor has a key role in mediating intracellular signaling by most of the TLRs, the sensitivity of MyD88-deficient mice to bacterial infection differs by the organism studied. For example, MyD88 is critical for TLR4 and TLR2 signaling, yet whereas MyD88-deficient animals fail to clear gram-negative *Pseudomonas aeruginosa* from the lungs and die within 24 h of inhalation challenge, the same MyD88-deficient animals clear the gram-positive *Staphylococcus aureus* normally (Figure 4) (24). More precise information is needed about the roles of the different Toll pathways in models of respiratory infection *in vivo*.

Figure 4. (A–D) Clearance of gram-positive and gram-negative bacteria from the lungs and spleens of mice deficient in the MyD88 intracellular adaptor protein, which mediates intracellular signaling from TLRs. Mice deficient in MyD88 had markedly impaired clearance of *Pseudomonas aeruginosa* from the lungs, and died 24 h after aerosol exposure, but survived the challenge with the gram-positive organism, *Staphylococcus aureus*. CFU = colony-forming units. Reprinted by permission from Reference 24.

INTERACTIONS BETWEEN INNATE IMMUNITY AND OTHER PATHWAYS

Innate immune pathways have important intersections with other intracellular pathways, such as the mechanotransduction pathways activated by cellular stretch, and apoptosis pathways triggered by membrane death receptors, but the molecular mechanisms involved are not completely clear. Mechanical stretch stimulates cytokine production by human alveolar macrophages *in vitro*, and simultaneous exposure to LPS enhances this effect (51). Similarly, rat lungs produce proinflammatory cytokines when ventilated with large tidal volumes *ex vivo*, and pretreatment of the animals with intravenous LPS enhances the proinflammatory response to any given level of stretch (52). This effect is demonstrable in anesthetized rabbits ventilated with moderately high tidal volumes (15 ml/kg), which might occur locally in damaged lungs, or with tidal volumes commonly used in humans without clinical lung injury (10 ml/kg) (53, 54). Whereas LPS sensitizes the lungs to the effects of mechanical stretch, additional evidence suggests that mechanical stretch can sensitize the lungs to bacterial products by enhancing innate immune pathways. When anesthetized rabbits were ventilated with large tidal volumes (20 ml/kg), CD14 expression increased in lung tissues, and the alveolar macrophages recovered by BAL had increased responsiveness to LPS *in vitro* (55). These bidirectional effects of mechanical stretch and LPS-dependent cellular activation raise the possibility that lessening mechanical stretch in the lungs and also blocking the effects of LPS are two complementary strategies to protect ventilated patients from lung injury. Consistent with this interpretation, the ARDSnet clinical trials have shown that reducing tidal volumes in humans with lung injury is associated with improved clinical outcome and with less systemic inflammation (56, 57). Manipulating innate immunity in humans at risk for ventilator-associated lung injury has not been tried.

Innate immunity pathways also intersect with receptor-mediated cell death pathways. Apoptosis can be triggered by a series of membrane receptors in the tumor necrosis factor receptor family, or by direct mitochondrial injury. The Fas pathway is activated when the membrane Fas receptor is clustered by Fas ligand on the surface of lymphocytes or soluble Fas ligand that is shed from the cell surface by the action of metalloproteinase, such as MMP-7 and others. The Fas pathway activates a sequence of caspases that lead to DNA cleavage and controlled cell death, but Fas pathway intermediates also are involved in NF- κ B activation in some cells (58). Mice with a naturally occurring inactivating mutation in Fas have a blunted proinflammatory response to intrapulmonary LPS (59). Overexpression of a key intracellular adaptor protein (Fas-associated death domain [FADD]) suppresses LPS-induced signaling through TLR-4 (60). Fas-associated death domain is known to associate with the intracellular portion of Fas, where it activates Fas-dependent apoptosis. In the current paradigm, FADD also can associate with the TLR4 adapter MyD88, and suppress TLR4-mediated signaling (58). Activation of Fas is believed to draw FADD away from the TLR4 signaling complex and enhance TLR4 signaling, so that death signals potentiate inflammatory signals in response to bacterial products.

DIVERSITY OF INNATE IMMUNE RESPONSES IN HUMANS

Innate immune responses are very diverse in humans. Clinicians realize that some patients tolerate relatively severe infections and respond promptly to antibiotics and supportive care, whereas others rapidly develop septic shock. It has been found consistently that there is a wide range of cytokine concentrations in the BAL fluid of patients with acute lung injury (39). Although the causes of acute lung injury are heterogeneous and the time when patients reach medical care after the onset of symptoms varies, inherent differences in the activation of innate immune pathways also are likely to be important. Wurfel and colleagues have used a simple assay in which whole blood is incubated with LPS or other bacterial products to create an intermediate cytokine response phenotype to study the distribution of innate immune responses in the normal population (61). By ranking individuals according to the production of a number of different proinflammatory cytokines, subgroups of consistently high or consistently low responders can be identified (Figure 5). Gene expression analysis identified clusters of common genes expressed in the high and the low responders to LPS. The high responders tended to activate regulators of cytokine production more rapidly, whereas the lower responders preferentially activated IFN-responsive pathways. In addition, there are strong suggestions that innate immune responsiveness is an inheritable trait, providing further support for the possibility that subjects with high or low responsiveness might be identifiable before the onset of illness. For example, the family members of patients with severe meningococcal sepsis who died were more likely to produce lower levels of TNF- α and higher levels of IL-10 when their blood was stimulated with LPS *ex vivo*, and point mutations in the TLR4 gene are linked with susceptibility to severe meningococcemia (62, 63). In a study of monozygotic and dizygotic twins, the heritability of LPS-induced cytokine responses exceeded 50% for TNF- α responses and 80% for IL-1 β responses (64).

INNATE IMMUNITY: UNANSWERED QUESTIONS

Great progress has been made in the last decade in understanding the molecular basis of innate immunity and the relevance of these discoveries for lung defenses. The important clinical

Figure 5. High- and low-response phenotypes in the human population using LPS as the defined stimulus in whole blood *ex vivo*. Peripheral venous blood from normal human volunteers was stimulated with LPS (10 ng/ml) for 6 h and cytokines were measured in the plasma supernatants. The results for each cytokine analysis were plotted in rank order and the dots for each subject were connected across all of the cytokines. Yellow lines denote consistently high responders, and blue lines denote consistently low responders. Other patterns are also visible in the background lines from the other subjects. IL = interleukin; TNF = tumor necrosis factor; MCP = monocyte chemoattractant protein. Reprinted by permission from Reference 61.

question now is whether strategies should be developed to manipulate innate immunity in the clinical setting. Until the late 19th century, inflammation was viewed as harmful, and pus formation at the site of a wound was a bad omen (2). Metchnikoff recognized that phagocytes might have a defensive role in ingesting and digesting microbes. Now it is recognized that innate immunity is critical in defending the host from microbial invasion, but at the same time it is known that innate immune reactions can directly and indirectly damage tissues. Striking the right balance at the right time is one of the great clinical challenges in managing innate immunity. The key questions are whether one should enhance or inhibit innate immunity, and if so, at what point in a clinical illness, particularly when the host was normal before the illness began.

Several strategies to enhance innate immunity have been tried in normal subjects, including using granulocyte colony-stimulating factor to increase the number and activation state of circulating neutrophils, and IFN- γ to enhance macrophage-dependent immunity. When granulocyte colony-stimulating factor was used to enhance immunity in nonneutropenic patients with community-acquired pneumonia, the number of circulating neutrophils increased dramatically, but there was no improvement in clinical outcome (65). Interestingly, this strategy did not increase the extent of lung injury that was seen, and the chest radiographs actually improved slightly faster in the treated patients. Granulocyte colony-stimulating factor did not improve the outcome of patients with pneumonia and severe sepsis (66). IFN- γ has been proposed as a treatment to improve the systemic inflammatory downregulation that occurs after the onset of sepsis. It has been shown to improve markers of macrophage activation in patients with sepsis, but beneficial effects on clinical outcome in humans have yet been proved (67). Direct manipulation of TLR responsiveness has not yet been tried in humans with serious infections. In a model of severe *E. coli* pneumonia and sepsis in rabbits, intravenous treatment with an anti-CD14 antibody improved systemic hemodynamics and reduced nitric oxide production, but it worsened lung bacterial clearance in non-antibiotic-treated animals (68). This experiment supports the paradigm that local innate immunity pathways are critical

for host responses to the bacterial infection, but that systemic innate immune responses are deleterious for the host. A reasonable strategy that merits testing is to use appropriate antibiotics to control bacterial growth at the local site of infection, and block systemic innate immune pathways to protect the systemic compartment of the host.

An additional strategy might be to inhibit innate immunity to prevent the sensitizing effects of innate immunity pathways on other stimuli, such as mechanical stretch in patients at risk for ventilator-induced lung injury. Because bacterial products, such as LPS, are commonly present in the lower airways of intubated patients (35), it might be possible to reduce the incidence or outcome of ventilator-induced lung injury by carefully reducing the responsiveness of lung innate immune pathways to aspirated bacterial products for a limited period of time. An antibody to TLR4 protects mice from the inflammatory effects of LPS in the lungs (69). The effect of this strategy on the synergistic effects of bacterial products and mechanical ventilation merits further study.

Effective innate immunity is critical for humans to resist the myriad microbes and microbial products encountered in daily life. The discovery of penicillin showed the lifesaving effects of using drugs to destroy bacteria. Whether further ground can be gained by manipulating innate immunity is an important question waiting to be answered.

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